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At the edge and on the top: molecular identification and ecology of *Daphnia dentifera* and *D. longispina* in high-altitude Asian lakes

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Abstract The occurrence of members of the highly diverse *Daphnia longispina* complex in Southern and Central Asian high-mountain lakes has been recognized for more than a century. Until now, however, no molecular data have been available for these populations inhabiting the "Roof of the World." Here, we present the first identification for *D.* gr. *longispina* from that region based on a molecular phylogeny. Our findings show that alpine lakes in the Pamir and Himalaya mountains host populations of widespread species of the complex, for which these are the highest known localities. A spineless morph from the Himalaya region, previously labeled as *D. longispina* var. *aspina*, was clustering tightly with *D. dentifera*, while a population from the Pamir mountain range was grouped with *D. longispina*. In addition, we analyzed ecological data available for lakes in the Khumbu region (Himalaya) to investigate ecological preferences of non-pigmented *D.* gr. *longispina*. The identified factors can at least partly be related to avoidance of high UV conditions by this species. We conclude that the widespread species *D. dentifera* and *D. longispina* also colonized the Asian high-mountain lakes, and identify the need for further research to trace the possible effect of rapid environmental changes in this region on the diversity and ecology of high-altitude *Daphnia* populations.

Keywords *Daphnia longispina* complex · Alpine lakes · Molecular systematics · UV radiation · 12S

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Introduction

Members of the *Daphnia longispina* complex are distributed throughout the Holarctic and comprise some of the most common and ecologically important water flea species (Petrusek et al., 2008). The different taxa within this complex are difficult to distinguish based on morphological characters, and frequent hybridization and introgression between several taxa further complicate a reliable morphological identification (Petrusek et al., 2008; Dlouhá et al., 2010). Within the last decades, several molecular methods have become available that allow to distinguish lineages and hybrids within the *D. longispina* complex (e.g., Wolf & Mort, 1986; Billiones et al., 2004; Brede et al., 2006; Skage et al., 2007). Most recent studies have mainly focused on ecology or diversity of a few widespread species and their hybrids inhabiting large lakes, such as *D. longispina* O. F. Müller, 1776, *D. galeata* G. O. Sars, 1863, and *D. cucullata* G. O. Sars, 1862, in Europe, and *D. galeata* and *D. dentifera* Forbes, 1891, in the Eastern Palearctic and North America.

However, other species with more restricted distributions occur in the Palearctic, for example, *D. lacustris*, mostly confined to Fennoscandia, but with some isolated occurrences in mountain lakes (Nilssen et al., 2007). Further, molecular methods have allowed distinguishing additional cryptic lineages in both Asia (Ishida et al., 2011; Zuykova et al., 2012) and Europe (Petrusek et al., 2008, 2012). At least one of these lineages has a very wide distribution as it has been found in the northeast of European Russia as well as in the Irkutsk Reservoir in Siberia, more than 3,000 km apart (Petrusek et al., 2012). Another distinct clade of the *D. longispina* complex, closely related to both *D. dentifera* and *D. longispina*, has been reported by Ishida & Taylor (2007a) from the Tomsk and Baikal regions of Siberia.

These findings accentuate the fact that the knowledge on the diversity of the *D. longispina* complex in Southern and Central Asia remains scarce as most of the available data are based on morphological identification only. Some older works (e.g., Sars, 1903; Uéno, 1937; Brehm & Woltereck, 1939) reported specific *Daphnia* morphs from this region, but linking these observations to presently recognized lineages is challenging. Nevertheless, without establishing such links, ecology and distribution of lineages only discovered by genetic analyses will also remain virtually unknown, and the wealth of data in the historic literature will be of limited use.

Despite the fact that mountain lakes are often important habitats for *Daphnia* (Manca et al., 1998; Winder & Spaak, 2001; Petrusek et al., 2007) and *Daphnia* may be important components of mountain lake food webs (e.g., Winder et al., 2003), these lakes are relatively understudied with respect to the diversity of *Daphnia* lineages they may contain. Various studies, however, provide evidence that many mountainous regions harbor a substantial number of endemic cladoceran taxa (e.g., Kotov et al., 2010; Van Damme & Eggermont, 2011) and that *Daphnia* populations, interesting from biogeographic (e.g., Petrusek et al., 2007) as well as evolutionary (e.g., Mergeay et al., 2008; Dufresne et al., 2011) perspectives, can be discovered in such environments. On the other hand, the finding of special daphniid lineages in alpine lakes is not a general rule. For example, an analysis of the *D. longispina* complex from lakes in eight mountain ranges of southeastern Europe revealed the presence of a single *Daphnia* species, though its populations were substantially differentiated (Hamrová et al., 2012).

Having obtained samples of *Daphnia* useful for genetic analyses from remote lakes of the mountain ranges of South and Central Asia (Himalaya and Pamir; Fig. 1), we therefore asked whether we can find cryptic lineages or rather the widely distributed members of the *D. longispina* complex in such habitats.

Daphnia longispina and similar forms have been reported from Central Asian mountains by numerous authors, for example, from lakes in Tibet and the Altai and Pamir mountains by Sars (1903), from Tibet by Daday (1908), from the Pamir by Vereschagin (1923) or Rylov (1930), and from the Chinese Khingan mountain range by Uéno (1937). Various forms from these regions have also been formally described, usually as specific varieties, but occasionally also as distinct species, e.g., *D. sonkulensis* Manuilova, 1964 from Tian-Shan mountains. Sars (1903) described four new varieties for *D. longispina* for Central Asia, among them, *D. longispina* var. *turbinata* from the Altai (later considered by some authors as a distinct species; e.g., Manuilova, 1964; Glagolev, 1995). In the description itself, Sars (1903) noted that var. *turbinata* was very close to certain forms of *D. lacustris*

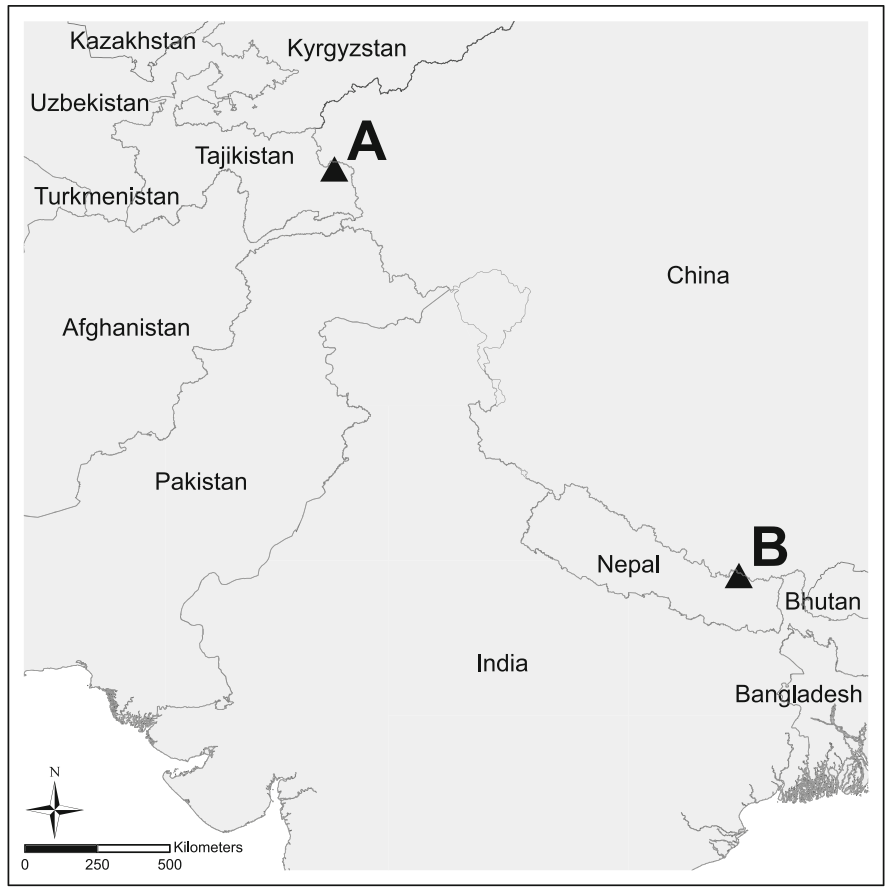

Fig. 1 Study locations (indicated by *triangles*) in Asian mountain regions (*A* Lake Rangkul, Pamir, *B* Lake Piramide Inferiore and Lake Gokyo, Khumbu region, Himalaya)

(considered by himself at that time as a subspecies of *D. longispina*). He also reported the *D. longispina* f. *caudata* from Tibet, a form that was shown to be mostly conspecific with *D. lacustris* in Fennoscandia (Nilssen et al., 2007). Several expeditions to North India and Tibet found a "pale" *D.* gr. *longispina*, often referred to as *D. longispina* or *D. longispina* var. *aspina* Vereschagin, 1911, and pigmented *Ctenodaphnia*, variously described as *D. tibetana* Sars, 1903, *D. fusca* Gurney, 1906, *D. pamirensis* Rylov, 1930, or *D. himalaya* Manca, Martin, Penalva-Arana & Benzie, 2006 (e.g., Hutchinson, 1937; Brehm & Woltereck, 1939; Löffler, 1969; Dumont & Velde, 1977; Manca et al., 1994).

A revision of the systematics of the pigmented *Ctenodaphnia* from this region is in progress (V. Kořínek, personal communication). Therefore, we focus here on the molecular systematics of the non-pigmented *D.* gr. *longispina*, presenting phylogenetic analyses for individuals collected from the Himalaya (Nepal) and the Pamir (Tajikistan) mountain ranges. We hypothesized that the distinctly different morphotypes collected in these two regions may represent different species.

In addition, we aimed to better understand the ecological factors driving the distribution patterns of non-pigmented *D.* gr. *longispina* in the harsh environment of the Himalaya range. Therefore, we collected and analyzed published data for lakes in the Khumbu region in northeastern Nepal (Manca et al., 1994, 1998; Tartari et al., 1998; Lacoul & Freedman, 2005; Sommaruga & Casamayor, 2009; Sommaruga, 2010; Sharma et al., 2012). These lakes are situated between 4,700 and 5,500 m a.s.l., are fishless, and are often inhabited by either non-pigmented *D.* gr. *longispina* and/or melanized

Ctenodaphnia. The daphnids frequently co-occur with the calanoid copepod *Arctodiaptomus jurisowitchi* Löffler, 1968, and various chydorid species (for further information, see Löffler, 1969; Manca et al., 1998; Tartari et al., 1998). Löffler (1969) already observed that the "pale" species seems to prefer turbid or deep lakes, whereas the pigmented one predominates in shallow and clear lakes, a pattern ascribed to differences in UV resistance. Furthermore, Sommaruga (2010) demonstrated the relevance of UV protection mechanisms for *A. jurisowitchi* in some of these lakes. However, there are many other factors that may potentially shape the distribution of different *Daphnia* species in high-elevation lakes besides UV stress, such as low temperatures, low nutrient/food concentrations, high suspended particulate matter content in lakes with glacier influence, and interspecific competition.

In the current study, we aimed to increase the knowledge on biogeography and ecology of the *D. longispina* complex by reconstructing the molecular phylogeny of two different morphotypes from the Pamir and Himalayan high-mountain lakes. Furthermore, we examined ecological preferences of *D.* gr. *longispina* in lakes of the Himalayan Khumbu region and discuss our findings also with respect to the ongoing environmental change in this habitat.

Materials and methods

Sampling sites and populations

Samples from the Himalaya region were collected from the Himalayan Lake Piramide Inferiore (LCN10, 27°57.8′N, 86°48.7′E, 5,067 m a.s.l.), Nepal (Khumbu region) on October 14, 2010. A detailed description of the sampling area is given in Tartari et al. (1998). Morphologically, individuals from this population fit the characteristics of *D. longispina* var. *aspina*, as reported from the Himalayan lakes (Fig. 2a, b). Phenotypically similar *Daphnia* is common in the region, inhabiting numerous alpine lakes (Löffler, 1969; Manca et al., 1998).

The population from Tajikistan was sampled on August 18, 2011 in the Pamir mountains from Lake Rangkul (38°28′N, 74°14′E, 3,789 m a.s.l.). Phenotypically, it seems close to *D. longispina* varieties *caudata* or *turbinata* as reported by Sars (1903) from Central Asia (see Fig. 2c). No other similar *Daphnia* was found in samples collected from smaller water bodies of the region (M. Slusarczyk & V. Kořínek, unpublished data). The samples were preserved in ethanol and thus suitable for DNA analyses.

Microphotography

The overall phenotype of the animals from the two regions was documented by microphotography. Specimens preserved in ethanol were stained after Kořínek (1999) with lignin pink and chlorazol black E dyes for 24 h, and subsequently either directly photographed or dehydrated with 2-2-dimethoxypropane for 10–15 min, then transferred into xylene, and mounted in Canada balsam. Sixty-four shots were taken at various focus plains by a Nikon D3100 camera mounted on an Olympus BX51 microscope with differential interference or phase contrast, and merged together using the software Helicon Focus 5.2.7. To document the Himalayan populations, better preserved individuals from Lake Gokyo (LCN75; 27°57′N, 86°41.5′E) were selected, which are both phenotypically and ecologically very close to those from Lake Piramide Inferiore (12 km apart).

Molecular analyses

DNA extraction from ethanol-preserved individuals and amplification of fragments of the 12S rRNA (12S) and NADH2 (ND2) genes as well as of the internal transcribed spacer region 1 (ITS-1) were done according to previously published protocols. DNA was extracted from five parthenogenetic females from the Himalayan sample following the HOTShot protocol (Montero-Pau et al., 2008) and from two individuals from Tajikistan using the proteinase K protocol as described by Schwenk et al. (1998).

A ca. 600 bp fragment of the mitochondrial 12S rDNA was amplified using primers and protocols from Taylor et al. (1996). Based on findings from the Eastern Palearctic by Ishida et al. (2011), we also tested for possible traces of past hybridization events and introgression in the Himalayan population. For this purpose, we additionally amplified a ca. 1,000 bp fragment of the mitochondrial ND2 gene and a ca. 760 bp one of the nuclear ITS-1 region using also the primers and protocols previously used in several studies for ND2 (Ishida et al., 2006; Ishida & Taylor,

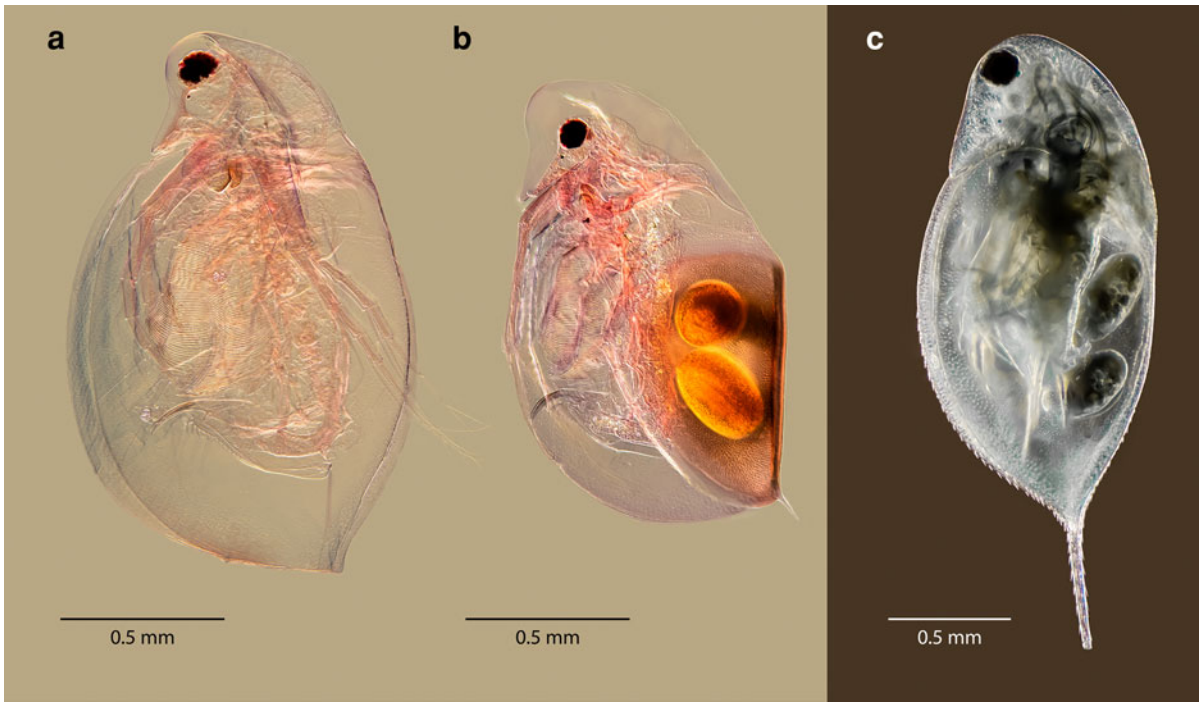

Fig. 2 Microphotographs of parthenogenetic (**a**) and ephippial (**b**) females of *D. dentifera* from Lake Gokyo, Khumbu region and of a parthenogenetic female of *D. longispina* (**c**) from Lake Rangkul, the Pamir mountain range. Photographs in **a** and **b** were taken using a differential interference contrast, the *right panel* (**c**) using a phase contrast

2007a, b) as well as for ITS-1 amplification (Taylor et al., 2005).

The PCR products were purified and Sanger-sequenced in both directions by commercial sequencing services (Microsynth AG, Switzerland, and Macrogen Inc., Korea). The chromatograms were checked visually for possible scoring errors, and the resulting sequences submitted to GenBank (accession numbers JX446618–JX446621). We obtained sequences of 562 bp fragments of the mitochondrial 12S gene for all analyzed individuals, and additionally a 937-bp-long fragment of the mitochondrial ND2 gene as well as a 762 bp fragment of the nuclear ITS-1 marker for the five individuals from the Himalayan locality.

The sequences of both mitochondrial genes were first compared with those representing known lineages of the whole *D. longispina* complex. For the final 12S alignment, selected sequences for *D. longispina* and *D. dentifera*, representing a wide range with respect to geographic distribution and genetic diversity, were used (Online Resource 1). The ND2 alignment was built from a subset of sequences used in Ishida & Taylor (2007a), kindly provided by Seiji Ishida, using *D. cucullata* and *D. galeata* (GenBank accession numbers: DQ980402 and DQ980251) as an outgroup. The ITS-1 sequences were aligned with the haplotypes published by Ishida et al. (2011). Alignment building, nucleotide substitution model selection, and phylogenetic tree reconstruction were done with MEGA 5 (Tamura et al., 2011).

Nucleotide substitution models were selected applying the Bayesian Information Criterion. The Tamura-Nei model of DNA evolution (Tamura & Nei, 1993) with gamma distributed rate heterogeneity was chosen for reconstruction of 12S and ND2 phylogenetic trees using the maximum likelihood (ML) method. Similarly, a ML tree was built for the ITS-1 sequences using the Kimura 2-parameter model (Kimura, 1980) with gamma distributed rate heterogeneity. In addition, a neighbor-joining (NJ) tree with evolutionary distances computed using the maximum composite likelihood method (Tamura et al., 2004) was constructed for each gene. The node support in each tree was assessed by 1,000 bootstrap replicates.

Statistical analyses of ecological data

A binary logistic regression analysis to identify factors explaining the presence of the non-pigmented *D.* gr.

longispina in lakes in the Himalayan Khumbu region was conducted using the statistical software R (R Development Core Team, 2012). Data from 20 lakes in this region were collected from several publications (Manca et al., 1994, 1998; Tartari et al., 1998; Lacoul & Freedman, 2005; Sommaruga & Casamayor, 2009; Sommaruga, 2010; Sharma et al., 2012). Variables collected were the presence/absence of the *D.* gr. *longispina* and the pigmented *Ctenodaphnia*, turbidity (milky/clear), water depth, lake area, altitude, electrical conductivity, and total concentrations of phosphorus (TP), silicate, and calcium. Logistic regression was computed using the function glm, stepwise model selection based on Akaike information criterion (AIC) was run with the function step, and likelihood ratio tests (LRT) for the significance of single terms in the selected models were done with the function drop1. Values for all quantitative variables were log-transformed to correct for positive skewness of their distributions.

Results

Identification of alpine *D.* gr. *longispina*

Based on the grouping of newly obtained sequences with those of known *Daphnia* species, we identified the individuals from the Himalayan locality (Fig. 2a, b) as *D. dentifera* and those from Tajikistan (Fig. 2c) as *D. longispina* sensu stricto. All individuals from the same location were identical in the analyzed genes.

The overall topologies of the phylogenetic trees based on the mitochondrial 12S (Fig. 3) and ND2 (Online Resource 2) genes are congruently supporting the existence of three closely related, but distinct, clades in Central Asia: *D. dentifera*, *D. longispina*, and a third clade from Siberia (Ishida & Taylor, 2007a). The phylogenetic reconstructions reflected different levels of variation present within these clades, the *D. dentifera* being most variable, with numerous distinct intraspecific lineages.

The nuclear ITS-1 phylogeny also identified the Himalayan sample as *D. dentifera*, and its congruent position in the nuclear and mitochondrial phylogenies revealed no indications for introgression or incomplete lineage sorting (Online Resource 3).

Fig. 3 ML phylogenetic tree for 12S rRNA. The new haplotypes from the Himalaya and Tajikistan are highlighted in *bold*. Numbers on major branches are bootstrap values (1,000 replications) obtained for ML and NJ phylogenetic analyses (ML/NJ). The *scale bar* indicates the number of substitutions per site ▶

Ecological preferences of Himalayan *D.* gr. *longispina*

The results of our statistical analysis suggest that the non-pigmented *D.* gr. *longispina* in the Khumbu region preferentially inhabit deep lakes at the lower range of total phosphorus concentrations and conductivity, and with glacier influence to some extent (i.e., the presence of minerogenic turbidity).

Model selection resulted in a best binary logistic regression model with depth, total phosphorus, turbidity, and conductivity as explanatory variables for the presence of the non-pigmented *D.* gr. *longispina* (LRT: $\chi^2 = 16.183$, df $= 4$, $P < 0.003$; Nagelkerke $R^2 = 0.629$; AIC: 21.34). According to the model, the likelihood for a lake being inhabited by the non-pigmented *D.* gr. *longispina* increases with greater depth and decreases with higher total phosphorus concentrations and conductivity. Lakes with some glacier influence were more likely to contain non-pigmented *D.* gr. *longispina*. Though minerogenic turbidity and especially conductivity were weakly significant, a model comparison based on LRT suggested that these parameters be retained in the model. Model parameters and LRT statistics are summarized in Table 1.

In the second best model, only depth and total phosphorus were kept as significant explanatory variables, with depth as the predictor of higher significance (LRT: $\chi^2 = 9.335$, df $= 2$, $P < 0.01$; Nagelkerke $R^2 = 0.377$; AIC: 24.19; see Table 2).

Discussion

Identification of alpine *D.* gr. *longispina*

The phylogenetic analyses revealed that *D.* gr. *longispina* from Lake Piramide Inferiore in the Nepalese Himalaya, formerly referred to as *D. longispina* var. *aspina*, actually constitutes a lineage within the *D. dentifera* clade. Furthermore, the 12S phylogeny clearly identified the individuals from Lake Rangkul

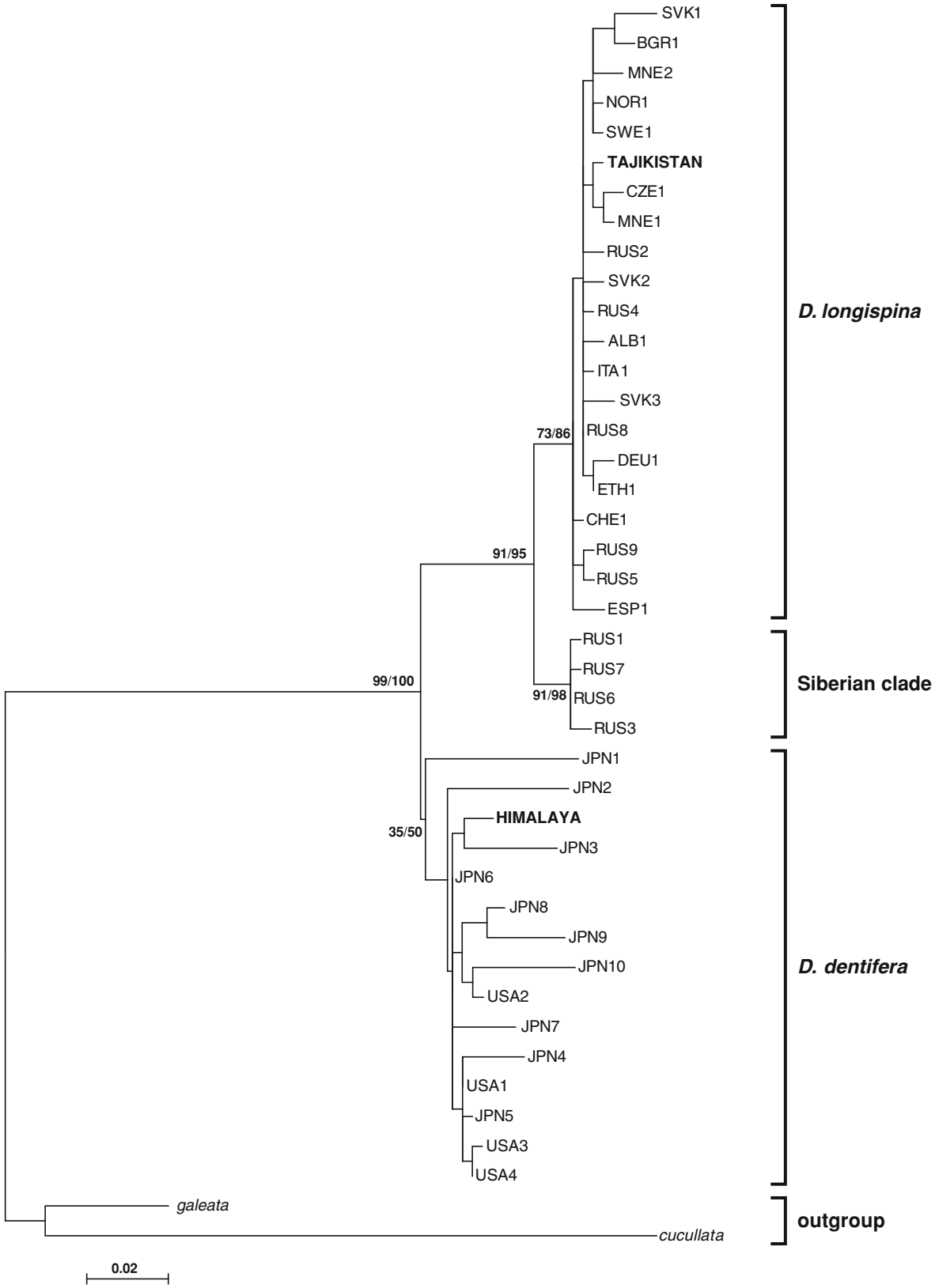
SVK1
BGR1
MNE2
NOR1
SWE1
TAJIKISTAN
CZE1
MNE1
RUS2
SVK2
RUS4
ALB1
ITA1
SVK3
RUS8
DEU1
ETH1
CHE1
RUS9
RUS5
ESP1
RUS1
RUS7
RUS6
RUS3
JPN1
JPN2
HIMALAYA
JPN3
JPN6
JPN8
JPN9
JPN10
USA2
JPN7
JPN4
USA1
JPN5
USA3
USA4
galeata
cucullata
73/86
91/95
99/100
91/98
35/50
0.02
D. longispina
Siberian clade
D. dentifera
outgroup

Table 1 Estimates and LRT statistics for a binary logistic regression model examining the absence/presence of the non-pigmented *Daphnia* in alpine lakes of the Khumbu region using water depth [log(Depth)], total phosphorus concentrations [log(TP)], the presence of minerogenic turbidity (clear/milky), and electrical conductivity [log(Cond)] as explanatory variables

	Estimate	Std. error	LRT	P ($>\chi^2$)
Intercept	22.018	12.649		
Log(Depth)	2.958	1.404	10.967	<0.001
Log(TP)	−11.546	5.772	10.791	0.001
Turbidity	9.721	5.353	6.766	0.009
Log(Cond)	−5.418	3.341	5.003	0.025

Null deviance: 27.526, df = 19; residual deviance: 11.343, df = 15

Table 2 Estimates and LRT statistics for a binary logistic regression model examining the absence/presence of non-pigmented *Daphnia* in alpine lakes of the Khumbu region using water depth [log(Depth)] and total phosphorus concentrations [log(TP)] as explanatory variables

	Estimate	Std. error	LRT	P ($>\chi^2$)
Intercept	0.120	1.705		
Log(Depth)	1.570	0.687	8.951	0.002
Log(TP)	−2.590	1.418	4.239	0.04

Null deviance: 27.526, df = 19; residual deviance: 18.190, df = 17

(Tajikistan) as *D. longispina*. We thus confirmed that the two morphotypes represented different species, but we revealed neither endemic nor rare species. On the contrary, both species are among the most widespread ones in the *D. longispina* complex, being confirmed genetically in at least two biogeographic regions each (Palearctic and Holarctic in the case of *D. dentifera*; Ishida & Taylor, 2007a; and Palearctic and Ethiopian in the case of *D. longispina*; Petrusek et al., 2008). Our results further extend our knowledge on the distribution of both species. To our knowledge, the Himalayan Lake Piramide Inferiore represents not only the southern- and westernmost, but also the highest locality reported for *D. dentifera*. Similarly, Lake Rangkul is the south-easternmost and also the highest known locality for *D. longispina*, evidenced by molecular data.

In detail, all three phylogenies, 12S, ND2, and ITS-1, grouped the Himalaya sample together with *D. dentifera* sequences from Japan and North America. We did not find evidence for past hybridization or introgression in our data (unlike in Japanese *D. dentifera* populations; see Ishida et al., 2011). It is likely that most or all populations with the *D. longispina* var. *aspina* morphotype that have been reported from several other lakes in the Khumbu region (Manca et al., 1994, 1998; Tartari et al., 1998) actually represent *D. dentifera*, and the mountain populations in the Himalayas do not coexist with other species of the same complex. However, given the high phenotypic plasticity and frequency of hybridization events in the *D. longispina* complex and the resulting difficulties for morphological identification, this prediction should be further tested by more detailed studies.

Although some of the morphs similar to animals from Lake Rangkul in the Pamir, reported by Sars (1903) from the Central Asian mountains, were considered by this author as close to *D. lacustris* (presently proven to be a distinct species), we unambiguously identified the Tadjik population as belonging to *D. longispina*. This species is particularly widespread in the European mountain ranges (Petrusek et al., 2007, 2008; Hamrová et al., 2012), and occurs in mountain lakes in numerous morphs that had been distinguished under separate names (as varieties and forms) in the early twentieth century (see e.g., Petrusek et al., 2007). It is thus possible that various varieties reported from Central Asia in historic literature are also conspecific with *D. longispina*.

However, it is undeniable that a wider diversity in the *D. longispina* complex can be found in this region, including lineages with unclear taxonomic status. A mitochondrial lineage closely related to both *D. longispina* and *D. dentifera* (based on ND2 analysis) has been reported from six widely distributed Siberian localities by Ishida & Taylor (2007a). Presumably, the same clade is apparent also in our 12S tree, represented by sequences from several localities in the Chany Lake basin in southwestern Siberia (Zuykova et al., 2012). Several other haplotypes from the Chany lake basin, however, can be found within the narrow *D. longispina* clade, indicating the coexistence of these two lineages in Siberia. Furthermore, other common species of the *D. longispina* complex, such as *D. galeata* and *D. cucullata*, are also distributed throughout Siberia (Ishida & Taylor, 2007b; Zuykova et al., 2010) and highly divergent members of the complex that cannot be at present linked to existing

names are found there as well (Petrusek et al., 2012; Zuykova et al., 2012). It is thus possible that some of these species also, at least occasionally, colonize high-altitude mountain lakes.

Ecological preferences of Himalayan *D.* gr. *longispina*

Another aspect of this work was the analysis of ecological data available for lakes in the Khumbu region to identify factors influencing the occurrence of the *D.* gr. *longispina* (identified as *D. dentifera* for Lake Piramide Inferiore in the present study). Logistic regression models on the presence/absence data for 20 lakes identified water depth, total phosphorus concentration (TP), minerogenic turbidity, and conductivity as significant predictors, with depth and TP being the most significant ones.

The finding that greater water depth increases the likelihood that the non-pigmented *D.* gr. *longispina* inhabits a lake is not surprising. In contrast to the melanized *Ctenodaphnia*, *D.* gr. *longispina* from this region do not contain photoprotective compounds (Sommaruga, 2010) in their body wall, except for pigmentation of the ephippia, and the importance of a deep water column as a refuge from ultraviolet radiation has been discussed in previous works already (Löffler, 1969; Manca et al., 1994, 1998; Sommaruga, 2001, 2010). In addition, deeper lakes are also better buffered against severe disturbances (e.g., freezing to the bottom, drying up) and might therefore constitute a more stable habitat in this harsh environment.

Interestingly, low TP was also a strong predictor for the presence of the *D.* gr. *longispina*. It is known for some members of the *D. longispina* complex that they can populate ultra-oligotrophic lakes, where they may even have a competitive advantages compared to other *Daphnia* species (Rellstab & Spaak, 2007; Spaak et al., 2012). However, the outcome of the statistical model did not change when including the most likely competitor in this system, the melanized *Ctenodaphnia*, as an additional variable. Actually, both pigmented and unpigmented *Daphnia* at least occasionally coexist in high-altitude Asian lakes (including e.g., Lake Rangkul in the Pamir from which we analyzed the *D. longispina* population), so it is clear that their encounter does not necessarily lead to competitive exclusion. It should also be mentioned that some of the measured TP values are close to the detection limit of the analytical method used. Consequently, the relevance of TP concentrations for *Daphnia* distribution in these lakes remains an open question for further research.

Löffler (1969) already emphasized the potential importance of turbidity for the occurrence of the non-pigmented *D.* gr. *longispina* in shallow ponds and attributed that observation to its protecting role from UV radiation. Nevertheless, it seems counterintuitive that substantial minerogenic turbidity from glaciers could be beneficial for *Daphnia* since several studies have shown the detrimental impact of high suspended particle concentrations on *Daphnia* (e.g., Koenings et al., 1990; Kirk, 1991; Rellstab & Spaak, 2007). The suspended particle concentrations measured in three lakes (Giardino et al., 2010) classified as turbid by Tartari et al. (1998) furthermore suggest that lakes with a relatively low minerogenic turbidity had been categorized as turbid or that turbidity has decreased in those lakes. Interestingly, the *D.* gr. *longispina* has also been reported from one shallow and transparent lake (LCN40, see Tartari et al., 1998), which was explained by the benthic growth of freshwater mosses or filamentous algae serving as a refuge for zooplankton (Manca et al., 1994, 1998, 2004). Similar observations have been made for the copepod *A. jurisowitchi* inhabiting a shallow high-altitude transparent pond colonized by filamentous algae (Sommaruga, 2010).

In summary, the factors identified by the model as important for *Daphnia* distribution can at least partly be attributed to UV stress and habitat stability. However, experimental research would be needed to elucidate their role.

Conclusion

The individuals from the *D. longispina* complex collected from Southern and Central Asia were identified as *D. longispina* and *D. dentifera* based on molecular phylogenies. That suggests that the distributional range of these species is considerably wider than assumed so far. Several factors explaining the presence of non-pigmented *D.* gr. *longispina* in high-altitude mountain lakes of the Khumbu region were identified. The significant effects of water depth and turbidity are well in accordance with previous studies, relating the presence of *D.* gr. *longispina* to conditions protecting from UV radiation. A shift in ecological

conditions due to the rapid retreat of glaciers in this region (Cruz et al., 2007) may potentially have negative impacts on *D.* gr. *longispina* populations. On the one hand, the increasing glacial run-off may increase minerogenic turbidity to levels that preclude the existence of *Daphnia* (Koenings et al., 1990). On the other hand, as soon as lakes lose their connection to the glacial drainage systems, their transparency and consequently UV stress for non-pigmented *Daphnia* may increase.

Altogether, the study has revealed that a limited number of samples from remote mountain areas can substantially improve our understanding of the phylogeny and distribution of *Daphnia* lineages. The fact that we found the widespread *D. dentifera* and *D. longispina* does not preclude the potential presence of cryptic lineages in this area. In the light of recent findings on the high cryptic lineage diversity in this complex (Petrusek et al., 2008, 2012; Ishida et al., 2011; Zuykova et al., 2012), investigating these mountain ranges seems to be even more interesting. Specifically, their remoteness and extreme habitats render the presence of locally adapted, so far undiscovered lineages of *Daphnia* as well as other cladocerans in these regions likely.

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